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Original Article

Alpha-Methylacyl-CoA Racemase Expression is Upregulated in Gastric Adenocarcinoma: A Study of 249 Cases

Camtu D. Truong[1], Wei Li[2], Wei Feng[2], Philip Cagle[3], Thaer Khoury[4], Sadir Alrawi[5], Keping Xie[6], James Yao[6] and Dongfeng Tan[1]

Departments of Pathology[1] and Gastrointestinal Oncology[6], The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; [2]The University of Texas Health Science Center at Houston, Houston, TX, USA; [3]The Methodist Hospital, Houston, TX, USA; [4]Roswell Park Cancer Institute, Buffalo, NY, USA and [5]University of Florida Health Science Center, Jacksonville, FL, USA

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Abstract: Alpha-methylacyl-CoA racemase (AMACR [P504S]) is a mitochondrial and peroxisomal enzyme involved in beta-oxidation of dietary branched-chain fatty acids and their derivatives. Recent studies showed that AMACR is expressed in several neoplasms, including prostate and colon cancer. However, AMACR expression in gastric neoplasms has yet to be thoroughly investigated. Because AMACR overexpression in human solid tumors is a potential target for cancer treatment, we aimed to evaluate the expression of AMACR in a large cohort of patients with gastric adenocarcinoma. The study evaluated 249 primary gastric adenocarcinomas by immunohistochemistry. Nonneoplastic gastric tissue samples from various sites (antrum, body, fundus, and pylorus) were also examined. The immunopositivity of each sample was graded on a scale from 0 to 3 (0, no expression; 1, weak expression, 2, intermediate expression; 3, strong expression). We observed AMACR expression in 141 tumor cases: 44, 47, and 50 cases had weak, intermediate, and strong expression, respectively. Both intestinal and signet ring cell adenocarcinoma cases had overexpression of AMACR, however intestinal adenocarcinoma had significantly higher expression than did signet ring cell adenocarcinoma ($p<0.05$). Nonneoplastic gastric mucosa did not show AMACR expression. The results of our study demonstrate that AMACR expression is upregulated in gastric cancer, and suggest that further prospective studies to explore the potential role of AMACR as a therapeutic target for gastric cancer are warranted.
Key Words: Gastric cancer, racemase, AMACR, immunohistochemistry, tissue microarray

Introduction

Gastric cancer is the second leading cause of cancer-related deaths and the fourth most common cancer world-wide. Gastric adenocarcinoma (GAC) has a dismal outcome since a high percentage of cases present with advanced disease, there is a lack of effective molecular targets, and gastric carcinogenesis is still poorly understood. Emerging evidence indicates that there are several convergent and interconnected signaling pathways that are involved in gastric carcinogenesis and are currently under active investigation. These are the mammalian target of rapamycin (mTOR) pathway, the Ras/Raf/Kinase/ERK pathway and the nuclear factor (NF)-κB pathway [5]. The mTOR pathway is known to regulate protein synthesis, cell-cycle progression, metabolism and angiogenesis [6]. It is regulated via sequential activation of multiple molecules, including alpha-methylacyl-CoA racemase (AMACR).

AMACR, also known as P504S, is a mitochondrial and peroxisomal enzyme that plays a crucial role in the beta-oxidation of bile acid intermediates, dietary branched-chain fatty acids, and fatty acid derivatives [1-4]. Recently, many investigators have reported the expression of AMACR in various tumors,

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including hepatocellular carcinoma, prostatic adenocarcinoma, pulmonary adenocarcinoma, papillary renal cell carcinoma, colorectal adenocarcinoma, and follicular thyroid carcinoma [1,5-12]. However, the expression of AMACR in gastric neoplasms is unknown. Because AMACR overexpression in human solid tumors is a potential target for cancer treatment, in this study, we aimed to evaluate the expression of AMACR in a large cohort of patients with gastric adenocarcinoma.

Materials and Methods

Patient Population

This study was performed with adherence to a protocol for investigation of molecular markers relevant to gastric cancer approved by The University of Texas M. D. Anderson Cancer Center Institutional Review Board. The criteria for inclusion in this study were a diagnosis of primary gastric adenocarcinoma, no treatment prior to complete surgical resection of the tumor, adequate archival tumor tissue samples available for analysis, and complete clinicopathologic data (age, sex, date of initial diagnosis, histopathologic diagnosis, tumor differentiation grade, nodal status, pathologic tumor stage, and date of death or last clinical follow-up examination) available on file. Two hundred forty-nine consecutive patients met the study criteria. The median follow-up duration was 57.8 months.

Histological Examination and Construction of Tissue Microarrays

Hematoxylin- and eosin-stained slides were reviewed to confirm the histopathologic diagnosis of gastric cancer followed by the selection of adequate tumor specimens for immunohistochemical analysis. Prior to the construction of tissue microarrays (TMAs), areas in tissue blocks containing adequate tumor were microscopically identified and marked by an investigator (D.T.). Thirty-eight nonneoplastic gastric tissue samples used as controls were included in the TMAs. Tissue cores 1 mm in diameter and 1-3 mm in length were taken from each donor block and arrayed into a new (recipient) block using a precision instrument (Beecher Instruments, Silver Spring, MD). For each case, three tissue cores of different areas of the tumor were sampled. The cores were spaced 0.8 mm apart in the new (recipient) block. A total of five high-density TMAs were used in this study.

Immunohistochemistry and Scoring of AMACR Expression

Immunohistochemical staining of 4-μm thick tissue sections cut from the TMA tissue blocks for AMACR was performed. Slides containing the sections were stained using an anti-AMACR monoclonal antibody kit according to the standard streptavidin-biotin-peroxidase procedure. The rabbit monoclonal antibody against P504S protein (clone 13H4, 1:40 dilution; Zeta Corporation, Sierra Madre, CA) was reacted with the tissue sections for 60 minutes at room temperature in a Dako automatic immunostainer (Dako, Carpinteria, CA) according to the manufacturer's instructions. Prostatic adenocarcinoma tissue sections were used as positive controls. Negative controls of prostatic adenocarcinoma were subjected to all the same reaction conditions except substituting water for the primary antibody. Expression of AMACR was indicated by a distinctive, coarse intra-cytoplasmic granularity. A scale of 0 to 3 was used to grade the expression, with 0 indicating no expression, 1 (up to 50% of cells with detectable staining) indicating weak expression, 2 (50% to 75% of cells with moderate staining) indicating intermediate expression, and 3 (more than 75% of cells with intense staining) indicating strong expression. The immunohistochemistry slides were independently reviewed by two pathologists. The highest expression score among three core samples was recorded for each case. Nonneoplastic gastric tissue samples (n=38) obtained from various sites (antrum, body, fundus, and pylorus) were also examined and used as controls.

Statistical Analysis

Mean values and standard deviations were calculated to describe the clinicopathologic variables including patient's age, gender, tumor histology, tumor grade, presence or absence of lymph node metastasis. Associations between the expression of AMACR and pathologic characteristics were analyzed using a *t*-test. The Cox proportional hazards regression model was used to estimate the relative risk of death and 95% confidence intervals associated with AMACR expression. A p value of less than 0.05 is considered statistically significant.

Table 1 Clinicopathologic features and AMACR expression of gastric carcinoma

		AMACR expression			P value
		Negative	Positive	Total (%)	
Gender	Female	25	56	81(37)	0.74
	Male	54	85	139(63)	
Age	<65	41	70	111(50)	0.63
	≥65	38	71	109(50)	
Lymph node	Negative	20	42	62(28)	0.27
	Positive	59	99	158(72)	
Type	WD/MD	18	61	79(36)	0.05
	PD	61	80	141(64)	
Stage	I or II	43	72	115(52)	0.28
	III or IV	36	69	105(48)	
Total		79	141	200(100)	

WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated

Results

The positive control samples demonstrated granular intracytoplasmic staining for AMACR in the neoplastic glands of prostatic adenocarcinoma. Of the 249 gastric carcinoma samples, 220 contained adequate tissue for interpretation after immuno-histochemical staining. 139 (63%) were male, and 81 (37%) were female. The median patient age was 65.1 years (range, 21.5-92.3 years). 69 of the gastric tumors (36%) were well or moderately differentiated, whereas 141 (64%) were poorly differentiated or undifferentiated. 115 patients had stage I or II disease, and 105 had stage III or IV disease. The clinicopathologic features of the patients are summarized in **Table 1**. Overall, we observed AMACR expression in 141 of the 220 tumor cases (64%). The results of immunohistochemical expression of AMACR in gastric adenocarcinoma are summarized in **Table 1**. Of the 141 tumor samples, 44 demonstrated weak expression (1+), 47 demonstrated intermediate expression (2+), and 50 demonstrated strong expression (3+). In contrast, none of the 38 nonneoplastic gastric mucosa samples had detectable expression of AMACR. Representative examples of AMACR expression in gastric cancer and non-neoplastic mucosa are illustrated in **Figures 1A-D**. The differential expression of AMACR in gastric cancer samples as compared to non-neoplastic gastric tissue is statistically significant ($p<0.05$).

Clinicopathologic parameters of patients with gastric carcinoma, including patient's age, gender, tumor histology, tumor grade, presence or absence of lymph node metastasis, were compared between groups of AMACR-positive and AMACR-negative gastric cancers. The association of expression of AMACR and the clinicopathologic variables are listed in **Table 2**. Both intestinal and signet ring cell adenocarcinoma samples had overexpression of AMACR. However, we observed significantly higher expression ($p<0.05$) in the intestinal adenocarcinomas as compared to the signet ring carcinomas. There was no association of expression of AMACR with other clinicopathologic parameters. The status of AMACR expression was not associated with patient's survival.

Discussion

In our cohort study of cases of gastric adenocarcinoma, we observed frequent overexpression of AMACR in gastric adenocarcinomas. Specifically, sixty-four percent (141/220) of gastric tumor samples displayed elevated expression of AMACR. By contrast, no expression of AMACR in normal gastric tissue was identified. The differential expression of AMACR in gastric cancer samples and non-neoplastic gastric tissue was statistically significant ($p<0.05$). The striking differential expression of AMACR in gastric cancer as compared to non-neoplastic gastric tissue has also been documented at mRNA level. For example, a recent study using quantitative real-time polymerase chain reaction analysis demonstrated robust AMACR mRNA expression in gastric carcinomas but

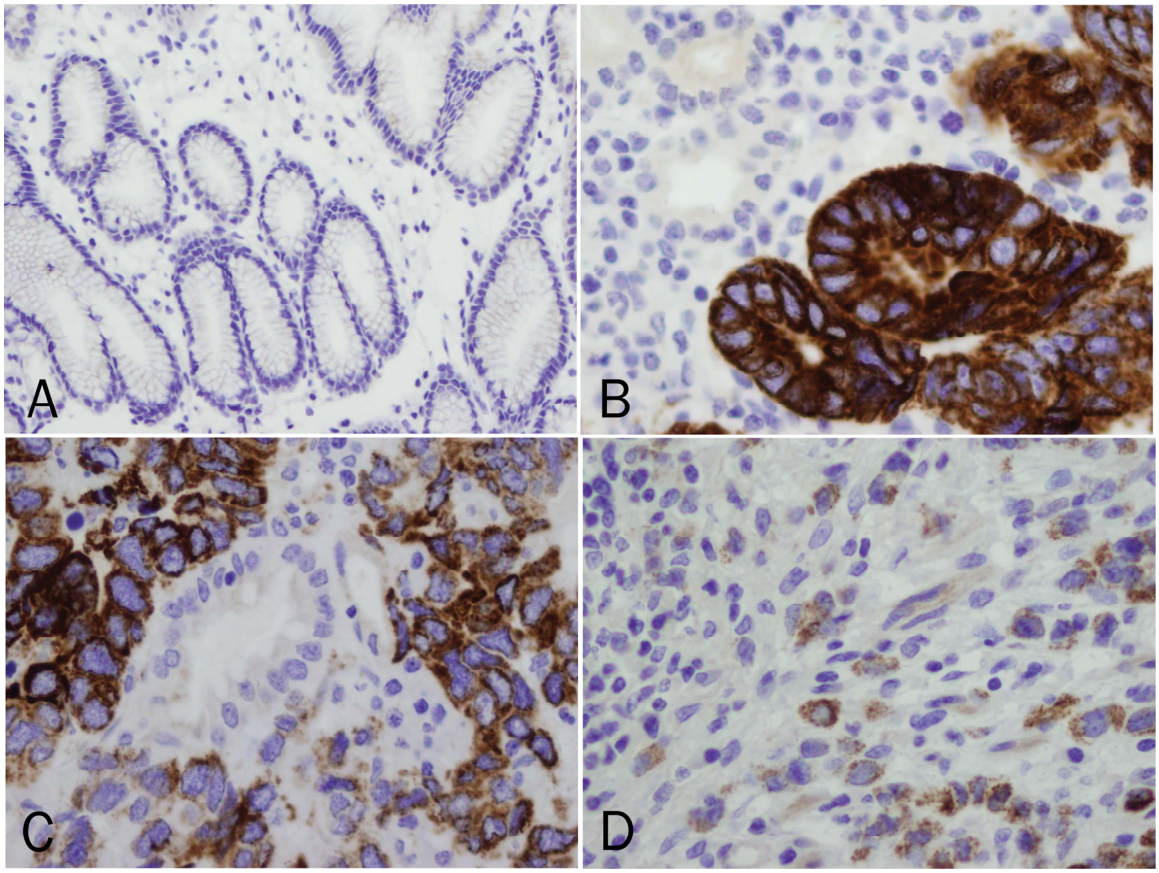

Figure 1 Representative AMACR expression in gastric tumor and non-neoplastic mucosa. **A**. No detectable AMACR expression in non-neoplastic mucosa. **B**. Well differentiated gastric adenocarcinoma with strong AMACR expression. Note the trapped gastric glands are negative for AMACR. **C**. Moderately differentiated gastric adenocarcinoma with moderate AMACR expression. Note the trapped gastric glands are negative for AMACR. **D**. Poorly differentiated gastric adenocarcinoma with weak AMACR expression. Note the background lymphocytes are negative for AMACR.

Table 2 Association of AMACR with clinicopathologic parameters

		RR	95% CI		P value
			Lower	Upper	
Gender	Female	Ref			
	Male	0.96	0.68	1.36	0.83
Age	<65	Ref			
	≥65	0.86	0.61	1.22	0.40
Lymph node	Negative	Ref			
	Positive	2.97	1.87	4.72	0.01
Type	WD/MD	Ref			
	PD	1.60	1.08	2.39	0.03
Stage	I or II	Ref			
	III or IV	2.14	1.51	3.03	0.01
AMACR	Negative	Ref			
	Positive	1.52	0.71	3.27	0.28

RR, relative risk; CI, confidence interval; WD/MD/PD, well/moderately/poorly differentiated

very low to undetectable levels of AMACR mRNA expression in normal gastric mucosa [20]. These observations suggest a potentially important function of AMACR in gastric adenoma/carcinoma tumorigenesis. The significant overexpression of AMACR in human gastric adenocarcinomas in our study further supports the role of upregulation of AMACR expression in gastric cancer development and progression.

In an independent study of AMACR in gastric cancer, Lee *et al* reported that AMACR was expressed in 52% (34/66) of cases of gastric carcinoma, 83% (40/48) of cases of gastric dysplasia, and 5% (2/44) of cases of nonneoplastic epithelium [13]. It is not clear why a minor subset of nonneoplastic gastric epithelium in their study displayed AMACR expression. One possible explanation is non-specific protein binding in gastric tissue, a known phenomenon due to its abundant endogenous enzymes. In addition, the authors used an AMACR antibody produced by a different biocompany, suggesting possible differences in epitope recognition or specificity. Our findings are more in agreement with those reported by Cho *et al* [14]. In their study, AMACR expression was identified in 63% (83/132) of cases of gastric adenocarcinoma, 79% (23/29) of cases of gastric adenoma, 8% (2/26) of cases of intestinal metaplasia, and 0% (0/32) of cases of normal gastric mucosa. Overall, we believe our data is fairly representative as it reflects a well-defined larger group of primary gastric adenocarcinoma that was tested in a uniform manner, namely, we simultaneously subjected all of our samples to the same sectioning, tissue microarray construction, and staining conditions, thus minimizing the preanalytical variables in the processes of the experiments. Nevertheless, additional validations of this finding, preferably among multiple institutions, will be needed to confirm the expression of AMACR in gastric adenocarcinoma and to evaluate its clinical significance. Of note, we observed significantly higher expression of AMACR in intestinal than in signet ring cell adenocarcinoma ($p<0.05$). This significant finding may indicate a role of AMACR in gastric tumor differentiation.

Gastric cancer is the second most common cause of cancer-associated deaths worldwide. The majority of patients with gastric cancer have advance-stage disease at the time of diagnosis. Radical surgical resection has been the main treatment modality for resectable disease [15-17]. However, up to 70% of patients with advance-stage gastric cancer have a relapse and die within 5 years after resection despite recent improvements in surgical treatment [15]. Recently, use of adjuvant chemotherapy and radiotherapy has led to reduced locoregional relapse rates, thus improving prognoses for gastric cancers. However, further improvements in local tumor control, reduction of metastasis, and minimization of therapy-related toxicity are still needed to increase the survival rates in patients with gastric cancer. Therefore, finding more specific targets for neoadjuvant therapy for gastric cancer is essential.

AMACR may be such a target. Emerging evidence suggests that more specific targets for combating gastric cancer are needed. Currently, AMACR's potential role as a target for treating gastric cancer seems to be promising. However, confirming this role requires a more thorough understanding of the function of AMACR in gastric tumorigenesis as well as its use as a therapeutic target. One possible function of AMACR in gastric cancer is via its ability to act as an activator of peroxisome proliferator-activated receptor (PPAR)-γ, an enzyme that is predominantly expressed in adipose tissue and has an important function in triggering adipocyte differentiation. Studies have shown that PPAR-γ is expressed in various human cancer cells, including colon, prostate, breast, and gastric cancer cells. Sato *et al* [18] reported strong expression of PPAR-γ in gastric carcinomas regardless of the tumor differentiation as well as PPAR-γ expression in gastric antral mucosa with intestinal metaplasia. Thus, AMACR may play a role in the promotion of gastric cancer cell growth through PPAR-γ activation [4, 18, 19].

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Please address all correspondences to Dongfeng Tan, MD, Department of Pathology, Unit 85, The University of Texas M. D. Anderson Cancer Center,

1515 Holcombe Blvd, Houston, TX, USA. Tel: 713-745-4977; Fax: 713-745-1105; Email: dtan@mdanderson.org

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